AMENDMENTS TO THE SPECIFICATION

Please replace paragraph beginning at page 10, line 26, with the following rewritten paragraph:

Figure 12A shows activation of MRGA receptors expressed in heterologous cells by neuropeptide ligands. HEK-$G\alpha_{15}$ cells (Offermanns and Simon. J Biol Chem *270*: 15175-80 (1995)) expressing MRGA1 were tested with the indicated ligands at a concentration of 1 μM. The data indicate the mean percentages of GFP-positive (i.e., transfected) cells showing calcium responses. None of the agonists indicated showed any responses through endogenous receptors in untransfected cells. Note that the RFamide neuropeptides FMRF (SEQ ID NO: 110), FLRF (SEQ ID NO: 111) and NPFF (SEQ ID NO: 113), as well as NPY, ACTH, CGRP-I and -II and somatostatin (SST) produced the strongest responses.

Please replace paragraph beginning at page 11, line 5, with the following rewritten paragraph:

Figure 12C shows the ligand selectivity of MRGA4. The data presented in Figures 12B and 12C indicate that the responses to the most effective ligands do not depend on the presence of $G\alpha_{15}$. Note that MRGA1-expressing cells respond to FLRF (SEQ ID NO: 111) and NPFF (SEQ ID NO: 113) but not to NPAF (SEQ ID NO: 112), while conversely MRGA4-expressing cells respond to NPAF (SEQ ID NO: 112) but not NPFF (SEQ ID NO: 113) or FLRF (SEQ ID NO: 111).

Please replace paragraph beginning at page 11, line 10, with the following rewritten paragraph:

Figure 12D shows dose-response curves for MRGA1 expressed in HEK-$G\alpha_{15}$ cells to selected RFamide neuropeptides. Each data point represents the mean ±S.E.M. of at least 3 independent determinations; at least 20 GFP^+ cells were analyzed for each determination. Responses at each ligand concentration were normalized to the maximal response subsequently shown by the same cells to a 5 μM concentration of FLRF (SEQ ID NO: 111). MRGA1 (D) shows highest sensitivity to FLRF (SEQ ID NO: 111) (squares, $EC_{50} \approx 20$ nM) and lower sensitivity to NPFF (SEQ ID NO: 113) (circles, $EC_{50} \approx 200$ nM).

Please replace paragraph beginning at page 11, line 17, with the following rewritten paragraph:

Figure 12E shows dose-response curves for MRGA4 expressed in HEK-Gα_{15} cells to selected RFamide neuropeptides. Each data point represents the mean ±S.E.M. of at least 3 independent determinations; at least 20 GFP$^+$ cells were analyzed for each determination. Responses at each ligand concentration were normalized to the maximal response subsequently shown by the same cells to a 5 μM concentration of NPAF (SEQ ID NO: 112). MRGA4 is preferentially activated by NPAF (SEQ ID NO: 112) (triangles, $EC_{50} \approx 60$ nM).

Please replace paragraph beginning at page 11, line 23, with the following rewritten paragraph:

Figure 12F shows dose-response curves for MAS1 expressed in HEK-Gα_{15} cells to selected RFamide neuropeptides. Each data point represents the mean ±S.E.M. of at least 3 independent determinations; at least 20 GFP$^+$ cells were analyzed for each determination. Responses at each ligand concentration were normalized to the maximal response subsequently shown by the same cells to a 5 μM concentration of NPFF (SEQ ID NO: 113). MAS1, like MRGA1, is activated by NPFF (SEQ ID NO: 113) with similar efficacy ($EC_{50} \approx 400$ nM), but is not as well activated by FLRF (SEQ ID NO: 111) (squares).

Please replace paragraph beginning at page 14, line 16, with the following rewritten paragraph:

The existence of a family of putative G protein-coupled receptors specifically expressed in nociceptive sensory neurons suggests that these molecules are primary mediators or modulators of pain sensation. It is therefore of great interest to identify ligands, both endogenous and synthetic, that modulate the activity of these receptors, for the management of chronic intractable pain. Indeed, ligand screens in heterologous cell expression systems indicate that these receptors can interact with RF-amide neuropeptides of which the prototypic member is the molluscan cardioexcitatory peptide FMRF-amide (SEQ ID NO: 114) (Price and Greenberg Science *197*: 670-671 (1977)).

Mammalian RF-amide peptides include NPFF (SEQ ID NO: 113) and NPAF (SEQ ID NO: 112), which are derived from a common pro-peptide precursor expressed in neurons of laminae I and II of the dorsal spinal cord (Vilim et al. Mol Pharmacol *55*: 804-11 (1999)). The expression of this neuropeptide FF precursor in the synaptic termination zone of *Mrg*-expressing sensory neurons, the ability of NPAF (SEQ ID NO: 112) and NPFF (SEQ ID NO: 113) to activate these receptors in functional assays, and the presence of binding sites for such peptides on primary sensory afferents in the dorsal horn (Gouarderes et al. Synapse *35*: 45-52 (2000)), together indicate that these neuropeptides are ligands for *Mrg* receptors *in vivo*. As intrathecal injection of NPFF (SEQ ID NO: 113)/NPAF (SEQ ID NO: 112) peptides produces long-lasting antinociceptive effects in several chronic pain models (reviewed in Panula et al. Brain Res *848*: 191-6 (1999)), including neuropathic pain (Xu et al. Peptides *20*: 1071-7 (1999)), these data further indicate that *Mrg*s are directly involved in the modulation of pain.

Please replace paragraph beginning at page 23, line 10, with the following rewritten paragraph:

By "Mrg ligand" is meant a molecule which specifically binds to and preferably activates an Mrg receptor. Examples of Mrg ligands include, but are not limited to RF-amide neuropeptides, such as FMRF (SEQ ID NO: 110), FLRF (SEQ ID NO: 111), NPAF (SEQ ID NO: 112), NPFF (SEQ ID NO: 113), and RFRP-1 for MrgA receptors, such as MrgA1. The ability of a molecule to bind to Mrg can be determined, for example, by the ability of the putative ligand to bind to membrane fractions prepared from cells expressing Mrg.

Please replace paragraph beginning at page 59, line 20, with the following rewritten paragraph:

As discussed in more detail below, several peptides have been putatively identified as endogenous ligands for Mrg receptors. In particular the RF-amide peptides, including NPAF (SEQ ID NO: 112) and NPFF (SEQ ID NO: 113), have been shown to efficiently stimulate several of the Mrg receptors. In order to identify additional new ligands for the Mrg receptors and ligands for drg-12, it is first necessary to identify ~~indetify~~ compounds that bind to these receptors. Thus, another embodiment of the

present invention provides methods of isolating and identifying binding partners or ligands of proteins of the invention. Macromolecules that interact with Mrg are referred to, for purposes of this discussion, as "binding partners." While the discussion below is specficially directed to identifying binding partners for Mrg receptors, it is contemplated that the assays of the invention may be used to identify binding partners for drg-12 as well.

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Please replace paragraph beginning at page 72, line 28, with the following rewritten paragraph:

Candidate Mrg agonist and antagonist small molecules are preferably first identified in an assay that allows for the rapid identification of potential agonists and antagonists. An example of such an assay is a binding assay wherein the ability of the candidate molecule to bind to the Mrg receptor is measured, such as those described above. In another example, the ability of candidate molecules to interfere with the binding of a known ligand, for example FMRFamide (SEQ ID NO: 114) to MrgA1, is measured. Candidate molecules that are identified by their ability to bind to Mrg proteins or interfere with the binding of known ligands are then tested for their ability to stimulate one or more biological activities.

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Please replace paragraph beginning at page 73, line 20, with the following rewritten paragraph:

Similar assays may also be used to identify inhibitors or antagonists of Mrg or *drg-12* activation. For example, cells expressing Mrg or *drg-12* and capable of producing a quantifiable response to receptor activation are contacted with a known Mrg or *drg-12* activator and the compound to be tested. In one embodiment, HEK cells expressing Gα15 and MrgA1 are contacted with FMRFamide (SEQ ID NO: 114) and the compound to be tested. The cellular response is measured, in this case increase in $[Ca^{2+}]$. A decreased response compared to the known activator by itself indicates that the compound acts as an inhibitor of activation.

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Please replace paragraphs beginning at page 75, line 28, with the following rewritten paragraph:

Identification of a neutralizing antibody involves contacting a cell expressing Mrg with a known Mrg ligand, such as an RF-amide peptide, and the candidate antibody and

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observing the effect of the antibody on Mrg activation. In one embodiment, Mrg receptors expressed in HEK cells overexpressing Gα15 are contacted with an Mrg ligand such as FMRFamide (SEQ ID NO: 114) and the candidate neutralizing antibody. A decrease in responsiveness to the ligand, measured as described in Example 5, would indicate that the antibody is a neutralizing antibody.

Please replace paragraphs beginning at page 102, line 14, with the following rewritten paragraphs:

The most efficient responses in *MrgA1*-expressing HEK cells were elicited by RFamide peptides, including FLRF (SEQ ID NO: 111) and the molluscan cardioactive neuropeptide FMRFamide (SEQ ID NO: 114) (Price and Greenberg Science *197:* 670-671 (1977)) (Phe-Met-Arg-Phe-amide) (Fig. 11C, 12A). Two mammalian RFamide peptides, NPAF (SEQ ID NO: 112) and NPFF (SEQ ID NO: 113), which are cleaved from a common pro-peptide precursor (Vilim et al. Mol Pharmacol *55*: 804-11 (1999)) were then tested. The response of *MrgA1*-expressing cells to NPFF (SEQ ID NO: 113) at 1 μM was similar to that seen with FMRFamide (SEQ ID NO: 114), while that to NPAF (SEQ ID NO: 112) was significantly lower (Fig. 12A). *MrgA1* was also weakly activated by two other RFamide ligands, γ_1-MSH and schistoFLRF (SEQ ID NO: 115) (data not shown).

In order to examine further the specificity of activation of *MrgA1* and *A4*, the top candidate ligands emerging from the intial screen were tested on these same receptors expressed in HEK cells lacking $G\alpha_{15}$. *MrgA1* and *A4* expressed in this system retained responses to RFamide peptides (Fig. 12B, C), demonstrating that the intracellular Ca^{2+} release responses seen in the initial screen are not dependent on the presence of exogenous $G\alpha_{15}$. This indicates that MrgAs act in HEK cells via Gq or Gi. The response of *MrgA1*-expressing HEK cells to NPFF (SEQ ID NO: 113) was lower than that to FLRF (SEQ ID NO: 111) (Fig. 12B), and there was no response to NPAF (SEQ ID NO: 112). Conversely, *MrgA4*-expressing cells responded to NPAF (SEQ ID NO: 112), but not to NPFF (SEQ ID NO: 113) or FLRF (SEQ ID NO: 111) (Fig. 12C). In both cases,

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the response to NPY seen in $G\alpha_{15}$-expressing cells (Fig. 11A) was lost completely, while those to CGRP-II and ACTH were considerably diminished.

Please replace paragraphs beginning at page 103, line 3, with the following rewritten paragraphs:

In order to determine the lowest concentrations of RFamide ligands capable of activating MrgA1 and A4, dose-response experiments were carried out in HEK cells expressing $G\alpha_{15}$, which afforded greater sensitivity (Fig. 12D, E). These experiments indicated that *MrgA1* could be activated by FLRF (SEQ ID NO: 111) at nanomolar concentrations (Fig. 12D; $EC_{50} \approx 20$ nM), and by NPFF (SEQ ID NO: 113) at about an order of magnitude higher concentration (Fig. 12D; $EC_{50} \approx 200$ nM), whereas NPAF (SEQ ID NO: 112) was much less effective. In contrast, *MrgA4* was well activated by NPAF (SEQ ID NO: 112) (Fig. 12E; $EC_{50} \approx 60$ nM), and much more weakly activated by FLRF (SEQ ID NO: 111) and NPFF (SEQ ID NO: 113). Neither receptor showed strong activation in response to RFRP-1, -2 or -3, a series of RFamide ligands produced from a different precursor (Hinuma et al. Nat Cell Biol *2*: 703-8 (2000)). These data confirm that *MrgA1* and *MrgA4* display different selectivities towards different RFamide ligands in this system. By contrast, these receptors responded similarly to ACTH (EC_{50} ~60- and 200 nM for *MrgA1* and *A4*, respectively; data not shown).

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Finally, given the sequence similarity between MRGA receptors and MAS1, the responsiveness of cells expressing exogenous *Mas1* to NPFF (SEQ ID NO: 113), NPAF (SEQ ID NO: 112) and FLRF (SEQ ID NO: 111) was tested. MAS1 showed a profile distinct from both *MrgA1* and *MrgA4* (Fig. 12F): like *MrgA1*, it was activated by NPFF (SEQ ID NO: 113) at a similar concentration of the peptide ($EC_{50} \approx 400$ nM), but unlike *MrgA1* it was poorly activated by FLRF (SEQ ID NO: 111). In contrast to *MrgA4*, MAS1 did not respond well to NPAF (SEQ ID NO: 112). No response was detected in MAS1-expressing cells upon exposure to Angiotensins I and II, ligands which have been previously reported to activate this receptor (Jackson, T. R., et al. Nature *335*: 437-40 (1988)). Nor did MAS1 respond to ACTH. Thus, MAS1, *MrgA1* and *MrgA4* expressed in this heterologous system are all activated by RFamide family ligands, but with differing ligand-sensitivities and -selectivities (Table 4).